

The Analysis of Fungal Pathogens *Pseudoperonospora humuli* and *Podosphaera macularis* as they affect the Hops Species *Humulus lupulus* and *Humulus japonicus*

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INTRODUCTION

The genus *Humulus*, commonly referred to as a hop, which belongs to the *Cannabaceae* family, is comprised of three species: *H. yunnanensis*, *H. japonicus*, and *H. lupulus* (Small, 1978). All three species of *Humulus* are dioecious (having separate male and female plants) perennials that climb in a clockwise manner, by using climbing hairs, or trichomes, located on the petioles (the stalk that attaches the leaf to the stem) and stems (Small, 1978). It is easy to determine the sex of a plant, upon flowering, because females produce “burrs” that develop into strobiles (cones) and males produce panicles (branching cluster of flowers) with 5 stamens, which contain pollen (Haunald, 2009). All species of *Humulus* produce basal spikes (shoots) that protrude from rhizomes (crowns), underground stems, and are able to spread via rhizomal growth, seed dispersal, and stem propagation (DeNoma, 2000, Haunald, 2009, and Neve, 1991).

H. lupulus is the only species of hops that has been commercially grown for its cones, which have been used to brew beer and ales since the Middle Ages (Patzak, 2005 and Neve, 1991). Small (1978) noted that there are five inter-fertile taxonomic subspecies, one native to Europe, one to eastern Asia and the remaining three in North America.

Domesticated hop plants have been historically grown for their production of α and β acids, which are present in the resins produced by glands in female cones. These compounds are useful in the brewing of beer as a preservative and flavoring agents (Gent, 2009). Recently other chemical products in the cones have become desirable to brewers, leading to a proliferation of *H. lupulus* varieties with different bitterness, flavor, and aroma characteristics. Also, another goal of modern breeding programs is to develop plants with high cone yield and resistance to agricultural disease and pests. Typical varieties of cultivated plants may grow up to 25 cm in one day and can reach heights up to 7 meters in one growing season; they are cultivated and maintained by using strings, wires, poles, or trellis systems in a “hop yard” (Haunold, 2009).

Typically, hop yards do not allow male plants to grow, because they are responsible for the pollination of the female plants and the creation of unwanted seeds. However, males are integral in the creation of new varieties, through controlled intra-

specific hybridization–breeding between genetically divergent individuals from the same species) (DeNoma, 2000).

H. japonicus, commonly referred to as Japanese hop, is a species of hop that is indigenous to Asia, where it has been used for ornamental purposes, such as hedges. Because its cones are smaller than those of *H. lupulus* and the low amount of resin producing glands on *H. japonicus*, the plant is not a viable resource for brewing (DeNoma, 2000). This species of hop has a strange genetic occurrence: the female plants are diploid ($2n = 16$) yet the male plant has an extra chromosome ($2n = 17$). Due to the previous information, *H. japonicus* cannot be hybridized with *H. lupulus* or *H. yunnanensis* (DeNoma, 2000 and Haunold, 2009). *H. japonicus* was imported to the United States in the late 1800s, to be used ornamentally. The National Park Service (2015) reports that the Japanese hop has become an invasive species in Connecticut, Delaware, Indiana, Maryland, Pennsylvania, Virginia, and the District of Columbia. This species of Hop can be found along streams and in floodplains, because the plant “prefers plentiful sunlight and moisture” (National Park Service, 2015). The term *invasive species* carries with it a negative connotation. For example, in the United States alone, they pose as threats to about 42% of Threatened or Endangered species (National Wildlife Federation, 2015). The Japanese hop is able to completely cover large areas of “bare ground, low vegetation, and small trees,” because they are able to grow fast in the summer months (National Park Service, 2015).

There is little known about *H. yunnanensis*; however, Small (1978) was able to identify a few key aspects of this species. *H. yunnanensis* is a dioecious, perennial plant with an unknown amount of chromosomes. He also marks that *H. yunnanensis* is misidentified as *H. lupulus*, yet, in all actuality, it resembles *H. japonicus*. Due to the few amount of glands in the bracteoles of the cones, “*H. yunnanensis* has value for brewing” (DeNoma, 2000).

Pseudoperonospora humuli is a fungal pathogen that causes hop downy mildew, which affects hop species worldwide by infecting “young shoots, leaves, flowers and cones, causing basal spikes, angular black leaf spots and brown discoloration of cones” (Patzak, 2005). This disease

is most prevalent in the Pacific Northwest, especially in the area of the Willamette Valley in Oregon, due to high amounts of rainfall; the disease is less prevalent in arid regions, such as the Yakima Valley of Washington (Chee, 2006). *P. humuli* establishes its infection in the crown of hop plants, from which “systemically infected shoots emerge in the spring and early summer” (Chee, 2006). Although *P. humuli* infection is present as soon as the shoot emerges from the crown of a hop plant, phenotypic expression of the disease does not come immediately, rendering the hop plant symptomless (Patzak, 2005). For this reason, Patzak (2005) used polymerase chain reaction (PCR) as a means to determine fungal infection, before symptoms were present.

Podosphaera macularis is a fungal pathogen that works as the agent for hop powdery mildew, a universal problem for hops (Patzak, 2005). Much like *P. humuli*, *P. macularis* causes the leaves and cones of plants to dilapidate, via bumps, blisters, pale spots, distortion of cones, and white mildew colonies (Patzak, 2005). In 1997, the same year that the fungal disease was discovered in the Pacific Northwest, *P. macularis* was held responsible for the demise of hop farming in New York (Gent, 2008).

Principal Approaches

In order to create a supply of hops, *H. japonicus* was located in the wild and the whole plant was taken out of the ground. The rhizomes from these plants were cut into 4-6 inch strips and planted in metromix and stored in a greenhouse. To promote growth in the greenhouse, each plant was connected via nylon string to a trestle. We also cut stems into 4-6 inch segments, each containing 1-2 leaves, and propagated them in 200 ml water; these tissue samples were stored in a growth chamber for a three week period at 25°C for 16 hours of light and 15°C for 8 hours of light. *H. lupulus var. lupulus* samples, including Cascade, Mount Hood, and Willamette, were collected from a residential estate in Scottsville, Virginia. An unknown subspecies of *H. lupulus* was obtained from the High Bridge Trail, located in Farmville, Virginia. Each subspecies of *H. lupulus* underwent the same stem propagation process as *H. japonicus*. Some of the plants that we grew in the greenhouse were subjected to outside sources of *Pseudoperonospora humuli* and *Podosphaera macularis* and others were left untouched by the fungal pathogens. Before we tampered with the plants, we ran a baseline PCR on each plant to determine the status of fungal infection.

In order to purposefully infect plants, we placed leaf samples with known *Pseudoperonospora*

humuli infections from Amherst County, Virginia and with unknown fungal infections from Maidens, Virginia. Leaves from these plants were used to infect plants in the greenhouse. To do so, an infected leaf was placed into a half opened ziplock bag and the bag enclosed a stem and leaf on an uninfected plant. In order to track the infection status, purposefully infected plants were compared to control plants that were not infected by humans.

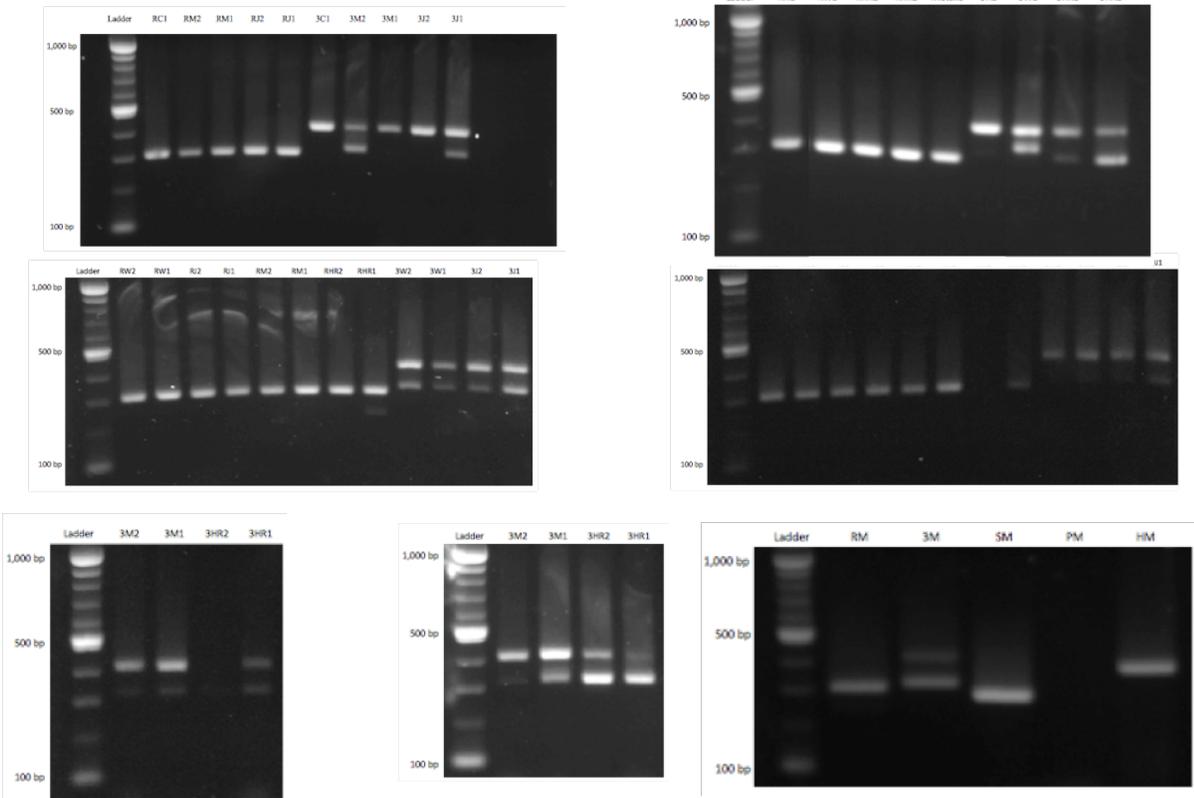
To extract plant DNA, we used a mortar and pestle to disrupt leaf tissue, in the bottom of a microcentrifuge tube, until a green liquid appeared. In place of a mortar and pestle, a tissue lyser may also be used; however, we found that a mortar and pestle wasn't a reliable source, but faster to use. Using the contents of a Qiagen DNEasy Plant Mini Kit, we added 400µl of Buffer AP1 and 4µl of RNase A to each of the disrupted plant tissue tubes and used a tabletop vortex to mix them for 10 seconds. Each tube was placed in a 65°C water bath for 10 minutes, inverting each tube 2-3 times. We then added 130µl of Buffer AP2 to each tube and vortexed them for 5 seconds, then incubated them for 5 minutes on ice. The tubes were centrifuged for 5 minutes at 14,000 rpm. Careful not to disturb the pellet formed at the bottom, we used a pipet to transfer 300µl of the liquid portion of each tube into a QIAshredder Mini spin column, placed into a 2ml collection tube. The tubes were centrifuged for 2 minutes at 14,000 rpm. We transferred the liquid flow-through into a new centrifuge tube, without disturbing the pellet at the bottom. Afterwards, 450µl of Buffer AP3/E was added to each tube and mixed by pipetting. We added 650µl of the mixture into a DNeasy Mini spin column in a 2 ml collection tube, centrifuged for 1 minute at 8,000 rpm, discarded the flow-through, and repeated this step with the remaining liquid from the AP3/E mixture. The DNeasy spin column was placed into a new 2 ml collection tube and 500 of µl Buffer AW was added; this mixture was then centrifuged for 1 minute at 8,000 rpm and the flow-through was discarded. Another 500 µl of Buffer AW was added to the spin column, centrifuged for 2 minutes at 14,000 rpm, before the spin column was carefully transferred to a new 2 ml microcentrifuge tube. Next, we added 100 µl of Buffer AE, for elution, and allowed each tube to sit for 5 minutes at room temperature before they were centrifuged for 1 minute at 8,000 rpm. The final step of the DNA extraction process was to remove the spin column, without making contact with the flow-through.

The extracted DNA was then used to run polymerase chain reaction (PCR), in order to determine fungal infection in the hops. In order to determine the status of fungal infection, the primers in

Figure 8 were used to amplify fragments of rDNA from *H. lupulus* and *H. japonicus*. To run PCR, 3µl of each primer from the pairs were added to a PCR tube, along with 10µl Mean Green Master Mix, 2µl sterile/deionized water, and 2µl template DNA. The PCR process was run based on the following steps: 95°C for 3:00, 95°C for 1:00, 55°C for 1:00, 72°C for

1:00, repeat steps 2 to steps 4 thirty-four times, 72°C for 5:00, 12°C for infinite hold. After the PCR process was complete, we ran the PCR products on a 2% agarose gel for 45 minutes.

Present Knowledge



Primer (Forward)	Primer (Reverse)	Nuclear rDNA Fragments (bp)	Species
R1 (GTGAACCGGCGGARGGATCATT)	R2 (TTYGCTRCGTTCTTCATCGATG)	305	<i>H. lupulus</i>
		297	<i>P. humuli</i>
		248	<i>P. macularis</i>
		204	<i>Verticillium albo-atrum</i>
R3 (GCATCGATGAGAACGYAGCRA)	R4 (TATCTTAARTTCAGCGGGT)	397	<i>H. lupulus</i>
		598	<i>P. humuli</i>
		312	<i>P. macularis</i>
		331	<i>V. albo-atrum</i>
S1 (CCCGAACTCATGTAGTTAGTGC)	S2 (GAGCACATCGGTACCGCCACTA)	282	<i>P. macularis</i>
P1 (CTGAGGGGACGAAAGGCTCTG)	P2 (CTGGTCACATGGACAGCCTCA)	371	<i>P. humuli</i>
HPMF2 (TGAAGCCACGCAGGGCGCCTGTC)	HPMR4 (ACTATGTTTAAFFACGCCGAA)	383	<i>P. macularis</i>

Graphs from left to right:
 Row 1: Picture 1 – Figure 1; Picture 2 – Figure 2
 Row 2: Picture 1 –Figure 3; Picture 2 – Figure 4
 Row 3: Picture 1 – Figure 5; Picture 2 – Figure 6; Picture 3 – Figure 7; Row 4: Figure 8: Primer Pairs

Figure 1: Baseline PCR tests of Cascade (C1), Mount Hood (M1 and M2), *H. japonicus* (J2 and J1) with primer pair R1 and R2 (R) and primer pair R3 and R4 (3).

Figure 2: Baseline PCR tests of High Bridge Trail (H1), Willamette (W1), High Bridge Trail rhizome (HR1 and HR2) with primer pair R1 and R2 (R) and primer pair R3 and R4 (3).

Figure 3: PCR detection of fungal infections, with primer pair R1 and R2 (R) and primer pair R3 and R4 (3), 1 week after contamination. All of the headings with a number two are the controls (no addition of infected leaves) and the headings with a number one underwent isolated contact with an infected leaf. Willamette (W1) and *H. japonicus* (J1) were contaminated with a *H. japonicus* leaf from Maidens, Virginia. Mount Hood (M1) and the High Bridge Trail rhizome (HR1) were both contaminated by a *H. lupulus* leaf from Amherst, Virginia that had a known *Pseudoperonospora humuli* infection.

Figure 4: PCR detection of fungal infections, with primer pair R1 and R2 (R) and primer pair R3 and R4 (3), 2 weeks after contamination. All of the headings with a number two are the controls (no addition of infected leaves) and the headings with a number one underwent isolated contact with an infected leaf. Willamette (W1) and *H. japonicus* (J1) were contaminated with a *H. japonicus* leaf from Maidens, Virginia. Mount Hood (M1) and the High Bridge Trail rhizome (HR1) were both contaminated by a *H. lupulus* leaf from Amherst, Virginia that had a known *Pseudoperonospora humuli* infection.

Figure 5: PCR detection of fungal infections, with primer pair R3 and R4 (3), 2 weeks after contamination. All of the headings with a number two are the controls (no addition of infected leaves) and the headings with a number one underwent isolated contact with an infected leaf. Mount Hood (M1) and High Bridge Trail rhizome (HR1) were contaminated by a *H. lupulus* leaf from Amherst, Virginia that had a known *Pseudoperonospora humuli* infection. High Bridge Trail rhizome (HR2) was contaminated by a *H. japonicus* leaf from Maidens, Virginia.

Figure 6: PCR detection of fungal infections, with primer pair R3 and R4 (3), 1 week after contamination. All of the headings with a number two are the controls (no addition of infected leaves) and the headings with a number one underwent isolated contact with an infected leaf. Mount Hood (M2) was contaminated with a *H. lupulus* leaf from Amherst, Virginia that had a known *Pseudoperonospora humuli* infection. High Bridge Trail rhizome (HR1) was

contaminated with a *H. japonicus* leaf from Maidens, Virginia

Figure 7: PCR detection of fungal infections, using primer pairs R1 and R2 (R), R3 and R4 (3), S1 and S2 (S), P1 and P2 (P), and HPMF2 and HPMR4 (H), in *H. japonicus* leaf from Maidens, Virginia.

The *H. lupulus* and *H. japonicus* rhizomes that were planted in the greenhouse grew at a much faster rate than the propagated stems; by the time the stems were ready to be planted, the rhizomes had already produced aboveground growth at heights near 12 inches.

One week after they were cut and placed into the growth chambers at 21°C, the propagated tissue did not show signs of healthy growth—many of the leaves began to wilt; the temperature in the chamber was then increased to 25°C. Two weeks after changing the chamber temperature, many of the propagated stems were ready to be planted. Of the *H. lupulus* subspecies, 21% of the unknown variety from the High Bridge Trail, 63% of Mount Hood, 4% of Cascade, and 40% of Willamette were successfully propagated. After two weeks of growth in the chamber, 58% of *H. japonicus* was successfully propagated.

Of the three High Bridge Trail specimens that were planted after propagation, all three of them grew to heights of over 6 ft. After ten of the Mount Hood specimens were planted in the greenhouse, only two of them were able to grow. After we planted one Cascade specimen, it was able to grow well and reached heights of over 6 ft. Six out of six of the Willamette specimens grew to heights of over 6 ft. The eleven *H. japonicus* specimens grew well in the greenhouse, all reaching heights of over 8 ft.

PCR primer pair R1 and R2 amplified a fragment of 300 bp nuclear rDNA from the *H. japonicus* from Maidens, Virginia. PCR primers R3 and R4 amplified fragments of 300 bp and 400 bp nuclear rDNA from the *H. japonicus* from Maidens, Virginia. PCR primer pairs S1 and S2 amplified a fragment of 280 bp nuclear rDNA from the *H. japonicus* from Maidens, Virginia. Primer pair P1 and P2 did not amplify any nuclear rDNA fragments from the *H. japonicus* from Maidens, Virginia. Primer pair HPMF2 and HPMR4 amplified a fragment of 380 bp nuclear rDNA from the *H. japonicus* from Maidens, Virginia (Figure 7).

As seen in Figure 1, the baseline PCR tests revealed that PCR primers R1 and R2 amplified a fragment of 300 bp nuclear rDNA regions from Cascade (C1), Mount Hood (M2 and M1), and *H. japonicus* (J2 and J1). PCR primers R3 and R4 amplified a fragment of 400 bp nuclear rDNA from

Cascade (C1), Mount Hood (M1), and *H. japonicus* (J2) and fragments of 300 bp and 400 bp nuclear rDNA from Mount Hood (M2) and *H. japonicus* (J1) (Figure 1). The baseline PCR tests revealed that PCR primers R1 and R2 amplified a fragment of 300 bp nuclear rDNA for the High Bridge Trail (H1), Willamette (W1), and the High Bridge Trail rhizome (HR1 and HR2) subspecies of *H. lupulus* (Figure 2). PCR primers R3 and R4 amplified a fragment of 400 bp nuclear rDNA from High Bridge Trail (H1) and fragments of 300 bp and 400 bp nuclear rDNA from High Bridge Trail rhizome (HR1 and HR2) (Figure 2). The previous primers also amplified fragments of 330 bp and 400 bp for Willamette (W1) (Figure 2).

One week after the baseline PCR data was collected and plants were contaminated, PCR primers R1 and R2 amplified a fragment of 300 bp nuclear rDNA for Willamette (W1 and W2), *H. japonicus* (J1 and J2), Mount Hood (M1 and M2) and High Bridge Trail rhizome (HR2) and fragments of 250 bp and 300 bp nuclear rDNA for High Bridge Trail rhizome (HR1)

(Figure 3). PCR primers R3 and R4 amplified fragments of 300 bp and 400 bp nuclear rDNA for Willamette (W1 and W2) and *H. japonicus* (J1 and J2) (Figure 3). PCR primers R3 and R4 amplified fragments of 300 bp and 400 bp nuclear rDNA for High Bridge Trail rhizome (HR1 and HR2) and Mount Hood (M1 and M2) (Figure 6).

Two weeks after the baseline PCR data was collected and plants were contaminated, PCR primers R1 and R2 amplified a fragment of 300 bp nuclear rDNA for Willamette (W1 and W2), *H. japonicus* (J1 and J2), Mount Hood (M1 and M2), and High Bridge Trail rhizome (HR1) (Figure 4). PCR primers R3 and R4 amplified a fragment of 400 bp nuclear rDNA for Willamette (W2) and fragments of 300 bp and 400 bp for Willamette (W1) and *H. japonicus* (J1 and J2) (Figure 4). PCR primers R3 and R4 amplified a fragment of 300 bp nuclear rDNA for High Bridge Trail rhizome (HR2) and fragments of 300 bp and 400 bp nuclear rDNA for Mount Hood (M1 and M2) and High Bridge Trail rhizome (HR1) (Figure 5).

DISCUSSION

The *H. lupulus* and *H. japonicus* rhizomes were able to grow at a faster pace than the propagated stems, because the root system of the rhizomes was able to collect nutrients at a much faster pace than the propagated stems. It would be of value to determine the sustainability and yield of the hops plants based upon the route of growth. We believe that rhizomal growth would produce healthier plants that are more resistant to fungal infections than propagated stem tissue; however, *P. humuli* and *P. macularis* spread via rhizomes.

We were surprised to find that of the *H. lupulus* species, the High Bridge Trail hops were not as successful as many of the other subspecies, with a 21% success rate, when it came to propagation. Strikingly, Mount Hood propagated with a 63% success rate, the highest of both the *H. lupulus* subspecies. It was, however, expected that the *H. japonicus* would be able to propagate with high success (58%), because it is an invasive species.

Although the propagation statistics did not follow our predictions, the plants grew in a fashion similar to our hypothesis. The High Bridge Trail and *H. japonicus* hops grew with a 100% success rate, because the plants were introduced to the climate of Virginia years ago. The Willamette and Cascade hops also grew with a 100% success rate, which was not expected. The Mount Hood hops did not grow with great success (20%), most likely due to a lack of altitude and an abundance of moisture.

The PCR primers R1 and R2, used to determine the fungal infection present in the *H. japonicus* leaf from Maidens, Virginia, provided some evidence that a *P. humuli* infection could be present;

however, this could be disputed because the banding pattern could be perceived as either 305 bp--*H. lupulus*--or 297 bp--*P. humuli*. For the sake of our research, we have determined the band to be 305 bp nuclear rDNA, which equates to a *H. lupulus* observation. The primers R3 and R4 provided evidence that *P. macularis* was infecting the plant from Maidens, Virginia. Primer pair S1 and S2 also provided evidence that the *H. japonicus* leaf was infected by *P. macularis*. Also, primer pair HPMF2 and HPMR4 proved to strengthen the notion that the leaf was infected by *P. macularis*.

The baseline PCR tests, for primers R1 and R2, amplified a fragment of 300 bp nuclear rDNA regions from Cascade (C1), Mount Hood (M2 and M1), and *H. japonicus* (J2 and J1), which indicated no sign of infection, showing only rDNA fragments from *H. lupulus* (Figure 1). Primers R1 and R2 showed no fungal infections for the High Bridge Trail (H1), Willamette (W1), or the High Bridge Trail rhizomes (HR1 and HR2) subspecies of *H. lupulus*, because a single band of 300 bp nuclear rDNA was present (Figure 2). PCR primers R3 and R4 amplified a fragment of 400 bp nuclear rDNA from Cascade (C1), Mount Hood (M1), and *H. japonicus* (J2), which constituted the presence of *H. lupulus* without an infection. Mount Hood (M2) and *H. japonicus* (J1) were determined to be infected by *P. macularis*, due to the presence of bands at 300 bp and 400 bp, even though primers R1 and R2 did not show an infection (Figure 1). PCR primers R3 and R4 amplified a fragment of 400 bp nuclear rDNA from High Bridge Trail (H1), resulting in a conclusion of no fungal infection, however, it seems that there may be a possible onset of *P. macularis* infection because of the light band at 300 bp nuclear rDNA (Figure 2). PCR primers R3 and R4 amplified a fragment of

roughly 330 bp nuclear rDNA from Willamette (W1); we believe this infection to be *V. albo-atrum* (Figure 2). The 300 bp and 400 bp nuclear rDNA fragments that were present in High Bridge Trail rhizome (HR1 and HR2) provided evidence that these hops were infected by *P. macularis*, even though primers R1 and R2 did not show an infection (Figure 2).

One week after the baseline PCR data was collected and plants were contaminated with leaves that were infected, PCR primers R1 and R2 showed no evidence of Willamette (W1 and W2), *H. japonicus* (J1 and J2), Mount Hood (M1 and M2), nor High Bridge Trail rhizome (HR2) to be infected (Figure 3). These primers did provide evidence that High Bridge Trail rhizome (HR1) was infected by *P. macularis*, which was no change from its fungal infection status during the baseline testing (Figure 3). PCR primers R3 and R4 showed a *P. macularis* infection in the leaf tissues of Willamette (W1 and W2), *H. japonicus* (J1 and J2), High Bridge Trail rhizome (HR1 and HR2), and Mount Hood (M1 and M2) (Figure 6); these results concluded that *H. japonicus* (J2) became infected by *P. macularis* without being contaminated by any infected leaves; hence, the plant must have been developing an infection that was present before it was propagated. Figure 3 shows Willamette (W1) with a *P. macularis* infection 1 week after being contaminated, instead of its previous *V. albo-atrum* infection. Mount Hood (M1) was free of infection before being contaminated by a leaf from Amherst, Virginia, which had a possible case of *P. humuli*, however, the PCR results show that the leaf developed a *P. macularis* infection, instead of *P. humuli* infection (Figure 6).

Two weeks after the baseline PCR data was collected and plants were contaminated with leaves that were infected, PCR primers R1 and R2 showed no sign of fungal infection in the rDNA of Willamette (W1 and W2), *H. japonicus* (J1 and J2), Mount Hood (M1 and M2), and High Bridge Trail rhizome (HR1) (Figure 4). In Figures 4 and 5, PCR primers R3 and R4 provided evidence that High Bridge Trail rhizome (HR2) and Willamette (W2) were not infected by *P. macularis*; this suggests that both plants were able to control the spread of the infection in a weeks time (Figure 4). PCR primers R3 and R4 provided evidence that *P. macularis* was present in Willamette (W1), *H. japonicus* (J1 and J2), Mount Hood (M1 and M2), and High Bridge Trail rhizome (HR1) (Figures 4, 5); the infection in Willamette (W1) and *H. japonicus* (J2) seemed to be controlling and decreasing the rate of the *P. macularis* infection (Figure 4)

Although many of the hop plants were contaminated with *P. humuli*--obtained from a *H. lupulus* leaf in Amherst, Virginia--and *P. macularis*--obtained from a *H. japonicus* leaf in Maidens,

Virginia--the hop plants were able to control the spread of the diseases. Some of the plants that were contaminated with *P. macularis*, such as Willamette (W1) and *H. japonicus* (J2), seemed to become less infected overtime. Figure 7 depicts four amplified nuclear rDNA bands, which code for a *P. macularis* infection, yet the leaves were not able to further infect any of the hop plants. Unfortunately, the primers R1 and R2 did not prove to be a reliable source of DNA information, because they only showed signs of infection one time. Furthermore, we are cautious to believe that the hop plants had large amounts of *P. macularis* and *P. humuli* in their systems. The large amount of data that we obtained by using primers R3 and R4 outweighs the notion that primers R1 and R2 did not yield sufficient infection results. We believe that there may have been a malfunction with the R1 and R2 primers, because the other primer pairs used in this research were able to amplify nuclear rDNA with the same results (Figure 7).

CONCLUSION

We would like to continue this research; so that we may better understand the time it takes a plant to become infected by *P. macularis* and *P. humuli*. Although *P. humuli* is of concern to the hop industry, we deem *P. macularis* to be a more beneficial fungi to study in the future, because it seems to be more prevalent and easier to detect in hops. Also, we would like to determine what caused the lack of amplification, via primers R1 and R2, because they could prove to be very reliable. Although primers S1 and S2, P1 and P2, and HPMF2 and HPMR4 were only used during one part of this study, it would be beneficial to use them more universally in future studies.

REFERENCES

- 1) Chee, H. Y., Nelson, M. E., Grove, G. G., Eastwell, K. C., Kenny, S. T., and Klein, R. E. 2006. Population biology of *Pseudoperonospora humuli* in Oregon and Washington. *Plant Dis.* 90:1283-1286.
- 2) China, Japan, South Korea trilateral summit. (2015, July 4). In *Wikipedia, The Free Encyclopedia*. Retrieved 13:42, July 16, 2015, from https://en.wikipedia.org/w/index.php?title=China%E2%80%93Japan%E2%80%93South_Korea_trilateral_summit&oldid=669882873
- 3) DeNoma, J.S., 2000. H. Genetic Resources. *USDA ARS National Clonal Germplasm Repository*.

<http://www.ars-grin.gov/cor/humulus/huminfo.html>

(July 14, 2015).

4) Gent, D. H., Nelson, M. E., George, A. E., Grove, G. G., Mahaffee, W. F., Ocamb, C. M., Barbour, J. D., Peetz, A., and Turechek, W. W. 2008. A decade of hop powdery mildew in the pacific northwest. Online. Plant Health Progress doi:10.1094/PHP-2008-0314-01-RV.

5) Gent, D.H., Nelson, M.E., Farnsworth, J.L., and Grove, G.G., 2009. PCR Detection of *Pseudoperonospora humuli* in air samples from hop yards. *Plant Pathology*, v. 58, pp. 1081-1091.

6) Haunold, A., 2009. Hops and Hop Growing: Soils, Plant Growth and Crop Production. *Encyclopedia of Life Support Systems (EOLSS)*, v. 2, pp. 1-4.

7) National Park Service, 2015. *H. japonicus*. Web. <http://www.nps.gov/plants/alien/pubs/midatlantic/huja.htm> (July15, 2015)

8) National Wildlife Federation, 2015. *Invasive Species*. Web. <https://www.nwf.org/Wildlife/Threats-to-Wildlife/Invasive-Species.aspx> (July 15, 2015)

9) Neve, R.A., 1991. *Hops*. Chapman and Hall, London, and Van Nostrand Reinhold Publishing Co, New York.

10) Patzak, J., 2005. PCR Detection of *Pseudoperonospora humuli* and *Podosphaera macularis* in *H. lupulus*. *Plant Protect Sci.*, v. 41. pp. 141-149.

11) Peetz, A.B., 2008. Understanding Sporulation and Dissemination of *Podosphaera macularis*, Hop Powdery Mildew. Oregon State University. Web. <http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/6492/Thesis%20Final.pdf?sequence>

12) Small, E., 1978. A Numerical and Nomenclatural Analysis of Morpho-Geographic Taxa of *H.*. *Systematic Botany*, v. 3, pp. 37-76.

13) USDA, NRCS. 2015. The PLANTS Database (<http://plants.usda.gov>, 15 July 2015). National Plant Data Team, Greensboro, NC 27401-4901 USA.